

IN THE CLAIMS:

Please cancel claims 12-18, 27, 28, 31 and 34, amend claims 19, 22, 29, 32, 33, 35 and 38, and add new claims 39-48.

This listing of claims will replace all prior versions, and listings of the claims in the application.

Listing of the claims

1.-18. (Canceled).

19. **(Currently amended)** A system for identifying a microRNA-~~recognition element~~ sequence of a microRNA that targets a selected mRNA sequence, wherein the microRNA that targets the selected mRNA sequence is a nucleic acid molecule that is 17-25 nucleotides, has a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element, and has a sequence that when paired with the selected mRNA sequence has free energy of -20 kcal/mole or less, the system comprising:

A) an input interface for inputting mRNA sequences, a database of mRNA sequences or a link for connecting to a remote data input interface, data or a database of mRNA sequences;

B) an input interface for inputting microRNA sequences, a database of microRNA sequences or a link for connecting to a remote data input interface, data or a database of microRNA sequences;

C) a processor programmed with instructions for performing a method of identifying a ~~comparing mRNA sequences to microRNA sequences to identify a microRNA recognition element according to sequence that targets a selected mRNA sequence;~~ the method of claims 12 comprising the steps of:

i) receiving input of identification of a selected mRNA sequence to be the target of the microRNA,;

ii) generating an oligonucleotide sequence that is 17-25 nucleotides and has complementarity to the selected mRNA sequence that is indicative of a microRNA for a microRNA-recognition element, wherein the oligonucleotide sequence includes

a proximal region that is 7-9 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 5' end which is the oligonucleotide's 5' terminus nucleotide,

a distal region that is 7-15 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 3' end which is the oligonucleotide's 3' terminus nucleotide, and

a loop region that is 0 nucleotides, 2-3 nucleotides, or 6-9 nucleotides, wherein when the loop region is 0 nucleotides, the 3' end of the proximal region is contiguous to the 5' end of the distal region, and when the loop region is 2-3 nucleotides or 6-9 nucleotides, the 3' end of the proximal region is contiguous to the 5' end of the loop region, and the 3' end of the loop region is contiguous to the 5' end of the distal region,

and wherein complementarity of the oligonucleotide sequence to the selected mRNA sequence that is indicative of a microRNA for a microRNA-response element is characterized by the selected mRNA sequence having a sequence that:

a) includes a region corresponding to the proximal region of the oligonucleotide sequence that is either: (1) completely complementary to the proximal region, or (2) has a single mismatch (x) to the 5' end of the proximal region, or (y) symmetrically placed between the 5' end of the proximal region and the 3' end of the proximal region; and

b) includes either (1) a region corresponding to the loop region of the oligonucleotide sequence that forms a loop of 2-5 non-paired nucleotides of the selected mRNA sequence when the loop region of the oligonucleotide sequence is 0, or (2) a region corresponding to the loop

region of the oligonucleotide sequence that has 0 nucleotides when the loop region of the oligonucleotide sequence is 6-9 nucleotides, or (3) a region corresponding to the loop region of the oligonucleotide sequence that has 2-3 nucleotides which form a bulge with 2-3 non-complementary nucleotides of mRNA when the loop region of the oligonucleotide sequence is 2-3 nucleotides; and

c) includes a region corresponding to the distal region of the oligonucleotide sequence that is either: (1) completely complementary to at least 7 contiguous nucleotides of the distal region of the oligonucleotide, including the 5' end of the distal region, or (2) contains (x) mismatches of 1-4 contiguous nucleotides and (y) matches of at least 5 nucleotides to a contiguous nucleotide sequence of the distal region of the oligonucleotide sequence, including the 5' end of the distal region;

iii) calculating the free energy of a pairing formed by the oligonucleotide sequence and the selected mRNA sequence, wherein a pairing of the oligonucleotide sequence and the selected mRNA sequence with free energy of -20 kcal/mole or less identifies the pairing as having free energy of a pairing of a microRNA and a microRNA recognition element; and

iv) identifying the oligonucleotide sequence that 1) has 17-25 nucleotides, 2) has a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA-recognition element for a microRNA and 3) has a sequence that when paired with the selected mRNA sequence has free energy calculated to be -20 kcal/mole or less,

wherein the oligonucleotide sequence identified as 1) having 17-25 nucleotide, 2) having a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA-recognition element for a microRNA and 3) having a sequence that when paired with the selected mRNA sequence has free energy calculated to be -20

kcal/mole or less is identified as a microRNA sequence of a microRNA that targets a selected mRNA sequence.

20. **(Previously presented)** The system of claim 19 comprising a link for connecting to a database of mRNA sequences.

21. **(Previously presented)** The system of claim 19 comprising an input interface for inputting microRNA sequences.

22. **(Currently amended)** A computer program embodied on a computer readable medium for implementation on a computer system that for identifying a microRNA-oligonucleotide sequence of a microRNA that targets a selected mRNA sequence,
wherein the microRNA that targets the selected mRNA sequence is nucleic acid molecule that is 17-25 nucleotides, has a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element and has a sequence that when paired with the selected mRNA sequence has free energy of -20 kcal/mole or less, and

the program comprising comprises instructions for performing the steps of the a method of claim 12 identifying a microRNA sequence of a microRNA that targets a selected mRNA sequence; said method comprising the steps of:

i) identifying the selected mRNA sequence to be targeted by the microRNA;
ii) generating an oligonucleotide sequence that is 17-25 nucleotides and has a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA for a microRNA-recognition element, wherein the oligonucleotide sequence includes

a proximal region that is 7-9 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 5' end which is the oligonucleotide's 5' terminus nucleotide,

a distal region that is 7-15 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 3' end which is the oligonucleotide's 3' terminus nucleotide, and

a loop region that is 0 nucleotides, 2-3 nucleotides, or 6-9 nucleotides, wherein when the loop region is 0 nucleotides, the 3' end of the proximal region is contiguous to the 5' end of the distal region, and when the loop region is 2-3 nucleotides or 6-9 nucleotides, the 3' end of the proximal region is contiguous to the 5' end of the loop region, and the 3' end of the loop region is contiguous to the 5' end of the distal region,

and wherein complementarity of the oligonucleotide sequence to the selected mRNA sequence that is indicative of a microRNA for a microRNA-response element is characterized by the selected mRNA sequence having a sequence that:

a) includes a region corresponding to the proximal region of the oligonucleotide sequence that is either: (i) completely complementary to the proximal region, or (ii) has a single mismatch (A) to the 5' end of the proximal region, or (B) symmetrically placed between the 5' end of the proximal region and the 3' end of the proximal region; and

b) includes either (i) a region corresponding to the loop region of the oligonucleotide sequence that forms a loop of 2-5 non-paired nucleotides of the selected mRNA sequence when the loop region of the oligonucleotide sequence is 0, or (ii) includes a region corresponding to the loop region of the oligonucleotide sequence that has 0 nucleotides when the loop region of the oligonucleotide sequence is 6-9 nucleotides, or (iii) includes a region corresponding to the loop region of the oligonucleotide sequence that has 2-3 nucleotides which form a bulge with 2-3 non-complementary nucleotides of mRNA when the loop region of the oligonucleotide sequence is 2-3 nucleotides; and

c) includes a region corresponding to the distal region of the oligonucleotide sequence that is either: (i) completely complementary to at least 7 contiguous nucleotides of the distal region of the oligonucleotide, including the 5' end of the distal region, or (ii) contains (A) mismatches of 1-4 contiguous nucleotides and (B) matches of at least 5 nucleotides to a contiguous nucleotide sequence of the distal region of the oligonucleotide sequence, including the 5' end of the distal region;

iii) calculating the free energy of a pairing formed by the oligonucleotide sequence and the selected mRNA sequence, wherein a pairing of the oligonucleotide sequence and the selected mRNA sequence with free energy of -20 kcal/mole or less identifies the pairing as having free energy of a pairing of a microRNA and a microRNA recognition element; and

iv) identifying the oligonucleotide sequence that 1) has 17-25 nucleotides, 2) has a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA-recognition element for a microRNA and 3) has a sequence that when paired with the selected mRNA sequence has free energy calculated to be -20 kcal/mole or less;

wherein the oligonucleotide sequence identified as 1) having 17-25 nucleotide, 2) having a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA-recognition element for a microRNA and 3) having a sequence that when paired with the selected mRNA sequence has free energy calculated to be -20 kcal/mole or less is identified as a microRNA sequence of a microRNA that targets a selected mRNA sequence.

23.-28. (Canceled).

29. (Currently amended) A method of preparing a microRNA that targets a selected mRNA sequence, the method comprising: ~~the steps of:~~

confirming that a microRNA candidate oligonucleotide functions to inhibit expression of a mRNA that comprises the selected mRNA sequence that is present in a cell, wherein the

microRNA candidate is a nucleic acid molecule that has 17-25 nucleotides, has a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element, and has a sequence that when paired with the selected mRNA sequence has free energy of -20 kcal/mole or less, and inhibition of expression of mRNA that comprises the selected mRNA sequence in a cell by the microRNA candidate oligonucleotide is confirmed by

contacting the microRNA candidate oligonucleotide with the mRNA that comprises the selected mRNA sequence that is present in the cell, and

determining expression of the mRNA that comprises the selected mRNA sequence in the cell;

wherein reduced expression of mRNA that comprises the selected mRNA sequence indicates that the microRNA candidate is a microRNA that targets the selected mRNA sequence; and,

wherein said microRNA candidate oligonucleotide was produced by a method comprising the steps of:

a) identifying a the selected mRNA sequence of the mRNA that is present in a the cell to be targeted by the microRNA candidate; wherein;

b) generating an oligonucleotide sequence ~~of a microRNA candidate oligonucleotide, wherein the oligonucleotide sequence that is~~

1) 17-25 nucleotides, and

2) has a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA-recognition element for a microRNA,

wherein the 17-25 nucleotides of the ~~microRNA candidate~~ oligonucleotide sequence include

i) a proximal region that is 7-9 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 5' end which is the ~~microRNA candidate's oligonucleotide sequence's~~ 5' terminus nucleotide,

ii) a distal region that is 7-15 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 3' end which is the ~~microRNA candidate's~~ oligonucleotide sequence's 3' terminus nucleotide, and

iii) a loop region that is 0 nucleotides, 2-3 nucleotides or 6-9 nucleotides,

wherein

when the loop region of the ~~microRNA candidate~~ oligonucleotide sequence is 0 nucleotides, the 3' end of the proximal region is contiguous to the 5' end of the distal region, and

when the loop region of the ~~microRNA candidate~~ oligonucleotide sequence is 2-3 nucleotides or 6-9 nucleotides, the 3' end of the proximal region is contiguous to the 5' end of the loop region and the 3' end of the loop region is contiguous to the 5' end of the distal region,

and

wherein complementarity of the oligonucleotide sequence to the selected mRNA sequence ~~to the microRNA candidate sequence~~ that is indicative of a microRNA for a microRNA-response element ~~for the microRNA candidate~~ is characterized by ~~an~~ the selected mRNA sequence having a sequence that:

i) includes a region ~~in the mRNA sequence~~ corresponding to the proximal region of the ~~microRNA candidate~~ oligonucleotide sequence that is either

A) completely complementary to the proximal region of the ~~microRNA candidate~~ oligonucleotide sequence, or

B) has a single mismatch to the 5' end of the proximal region of the ~~microRNA candidate~~ oligonucleotide sequence, or

C) has a single mismatch symmetrically between the 5' end of the proximal region of the ~~microRNA-candidate~~ oligonucleotide sequence and the 3' end of the proximal region of the ~~microRNA-candidate~~ oligonucleotide sequence;

ii) includes a region ~~in the mRNA sequence~~ corresponding to the loop region of the ~~microRNA-candidate~~ oligonucleotide sequence that either

A) forms a loop of 2-5 non-paired nucleotides of mRNA when the loop region of the ~~microRNA-candidate~~ oligonucleotide sequence is 0, or

B) has 0 nucleotides of mRNA sequence when the loop region of the ~~microRNA-candidate~~ oligonucleotide sequence is 6-9 nucleotides, or

C) has 2-3 nucleotides in the mRNA sequence which form a bulge of 2-3 non-complementary nucleotides in the selected mRNA sequence when the loop region of the ~~microRNA-candidate~~ oligonucleotide sequence is 2-3 nucleotides; and

iii) includes a region corresponding to the distal region of the oligonucleotide sequence that is either:

(A) completely complementary to at least 7 contiguous nucleotides of the distal region of the ~~microRNA-candidate~~ oligonucleotide sequence including the 5' end of the distal region of the ~~microRNA-candidate~~ oligonucleotide sequence, or

(B) contains (i) mismatches of 1-4 contiguous nucleotides of the selected mRNA sequence and (ii) matches of at least 5 nucleotides of the selected mRNA sequence to a contiguous nucleotide sequence of the distal region of the ~~microRNA-candidate~~ oligonucleotide sequence including the 5'

end of the distal region of the ~~microRNA candidate~~
oligonucleotide sequence;

c) determining free energy of a pairing of the oligonucleotide sequence and the selected mRNA sequence to identify the pairing as having free energy of a pairing of a microRNA and a microRNA recognition element, wherein a pairing of the oligonucleotide sequence and the selected mRNA sequence has of free energy of -20 kcal/mole or less identifies the pairing as having free energy of a pairing of a microRNA and a microRNA recognition element; and

e) d) synthesizing a nucleic acid molecule comprising the oligonucleotide sequence that has a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA-recognition element for a microRNA, and that has a sequence that when paired with the selected mRNA sequence was determined to have free energy of -20 kcal/mole or less, wherein the nucleic acid molecule comprises a microRNA candidate oligonucleotide, ~~having said oligonucleotide sequence.~~

30. **(Previously Presented)** The method of claim 29 wherein contacting the microRNA candidate oligonucleotide with the selected mRNA present in the cell results in inhibition of the expression of the mRNA by at least 10%.

31. **(Canceled)**

32. **(Currently amended)** The A method of ~~claim 12~~, preparing a microRNA comprising the steps of:

- i) identifying a selected mRNA sequence to be the target of the microRNA;
- ii) generating an oligonucleotide sequence of a microRNA that is 17-25 nucleotides and has a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA-recognition element for the microRNA wherein the microRNA includes

a proximal region that is 7-9 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 5' end which is the microRNA's 5' terminus nucleotide,
a distal region that is 7-15 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 3' end which is the microRNA's 3' terminus nucleotide, and
a loop region that is 6-9 nucleotides,
wherein the 3' end of the proximal region is contiguous to the 5' end of the loop region, and the 3' end of the loop region is contiguous to the 5' end of the distal region,
wherein-in-step complementarity of the selected mRNA sequence to the microRNA sequence that is indicative of a microRNA-response element for the microRNA is characterized by:

the selected mRNA sequence having a sequence that:

a) includes a-region corresponding to the proximal region of the microRNA that is either: (i) completely complementary to the proximal region, or (ii) has a single mismatch (A) to the 5' end of the proximal region, or (B) symmetrically placed between the 5' end of the proximal region and the 3' end of the proximal region; and

b) ~~the mRNA~~ includes a region corresponding to the loop region of the microRNA, wherein said region of the selected mRNA has 0 nucleotides; ~~when the loop region of the microRNA is 6-9 nucleotides and~~

c) includes a region corresponding to the distal region of the microRNA that is either: (i) completely complementary to at least 7 contiguous nucleotides of the distal region of the microRNA, including the 5' end of the distal region, or (ii) contains (A) mismatches of 1-4 contiguous nucleotides and (B) matches of at least 5 nucleotides to a contiguous nucleotide sequence of the distal region of the microRNA, including the 5' end of the distal region;

wherein the oligonucleotide sequence has a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA for a microRNA-recognition element; and

iii) synthesizing a nucleic acid molecule, wherein the nucleotide sequence of the nucleic acid molecule is the microRNA oligonucleotide sequence.

33. (Currently amended) A method of preparing a microRNA that targets a selected mRNA sequence,

wherein the microRNA that targets the selected mRNA sequence is a nucleic acid molecule that is 17-25 nucleotides, has a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element and has a sequence that when paired with the selected mRNA sequence has free energy of -20 kcal/mole or less,

the method comprising the steps of:

I) identifying a selected mRNA sequence to be the target of the microRNA;

II) generating a microRNA an oligonucleotide sequence that:

A) is 17-25 nucleotides, wherein the selected mRNA sequence has a degree of complementarity to the microRNA oligonucleotide sequence that is indicative of a microRNA recognition element for a microRNA having the microRNA oligonucleotide sequence, wherein the microRNA oligonucleotide sequence and includes:

a) a microRNA proximal region that is 7-9 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 5' end which is the microRNA's 5' terminus nucleotide,

b) a microRNA distal region that is 7-15 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 3' end which is the microRNA's 3' terminus nucleotide, and

c) a microRNA loop region that is 0 nucleotides, 2-3 nucleotides, or 6-9 nucleotides,

wherein when the microRNA loop region is 0 nucleotides, the 3' end of the microRNA proximal region is contiguous to the 5' end of the microRNA

distal region, and when the microRNA loop region is 2-3 nucleotides or 6-9 nucleotides, the 3' end of the microRNA proximal region is contiguous to the 5' end of the microRNA loop region, and the 3' end of the microRNA loop region is contiguous to the 5' end of the microRNA distal region, and

B) has complementarity with the selected mRNA sequence that is indicative of a microRNA for a microRNA-response element, wherein complementarity of the oligonucleotide sequence to the selected mRNA sequence to the microRNA oligonucleotide sequence that is indicative of a microRNA for a microRNA-response element for the microRNA is characterized by: an a selected mRNA sequence having a sequence that:

a) includes an mRNA region corresponding to the microRNA proximal region of the microRNA that is either:

(i) completely complementary to the microRNA proximal region,
or

(ii) has a single mismatch

(A) to the 5' end of the microRNA proximal region, or

(B) symmetrically placed between the 5' end of the microRNA proximal region and the 3' end of the microRNA proximal region; and

b) includes an mRNA region corresponding to the microRNA loop region of the microRNA that either forms an mRNA loop of 2-5 non-paired nucleotides of mRNA when the microRNA loop region of the microRNA is 0, or the mRNA region corresponding to the microRNA loop region of the microRNA has 0 nucleotides when the microRNA loop region of the microRNA is 6-9 nucleotides, or the mRNA region corresponding to the microRNA loop region of the microRNA has 2-3 nucleotides which forms a bulge of 2-3 non-complementary nucleotides of mRNA when the microRNA loop region of the microRNA is 2-3 nucleotides; and

c) includes an mRNA region corresponding to the microRNA distal region of the microRNA that is either:

(i) completely complementary to at least 7 contiguous nucleotides of the microRNA distal region of the microRNA, including the 5' end of the microRNA distal region, or

(ii) contains

(A) mismatches of 1-4 contiguous nucleotides and

(B) matches of at least 5 nucleotides to a contiguous nucleotide sequence of the microRNA distal region of the microRNA, including the 5' end of the microRNA distal region;

III) determining free energy of a ~~microRNA having pairing of the microRNA~~ oligonucleotide sequence ~~bound to and the selected mRNA sequence to identify the pairing as having free energy of a pairing of a microRNA and a microRNA recognition element,~~ wherein a free energy determination of ~~-10~~ -20 kcal/mole or less indicates that said selected mRNA sequence is a microRNA-recognition element for ~~the~~ a microRNA having the ~~microRNA~~ oligonucleotide sequence; and

IV) synthesizing a nucleic acid molecule, ~~wherein the nucleotide comprising the 17-25 nucleotide oligonucleotide sequence of the~~ that has complementarity with the selected mRNA sequence that is indicative of a microRNA for a microRNA-response element and that has a sequence that pairs with selected mRNA sequence with a free energy determined to be -20 kcal/mole or less, wherein a nucleic acid molecule ~~having is the microRNA 17-25 nucleotide oligonucleotide sequence that has complementarity with the selected mRNA sequence that is indicative of a microRNA for a microRNA-response element and that has a sequence that when paired with the selected mRNA sequence has free energy determined to be -20~~ kcal/mole or less is a microRNA that targets the selected mRNA sequence.

34. (Canceled)

35. **(Currently amended)** The method of claim 33 wherein nucleic acid molecule synthesized in step IV comprises a microRNA oligonucleotide sequence that has a sequence that pairs with selected mRNA sequence with a free energy ~~determination of determined to be~~ -30 kcal/mole or less.

36. **(Previously Presented)** The method of claims 33 wherein the selected mRNA sequence is in the 3' untranslated region of an mRNA.

37. **(Previously Presented)** The method of claim 33 further comprising the step of testing the function of the oligonucleotide to determine whether the oligonucleotide modulates the expression of the mRNA.

38. **(Currently amended)** The method of claim 33, wherein the step of testing the function of the microRNA results in ~~activation or~~ inhibition of the expression of the mRNA by at least 10%.

39. **(New)** The method of claim 33, wherein the step of testing the function of the microRNA results in inhibition of the expression of the mRNA by at least 20%.

40. **(New)** The method of claim 33, wherein the step of testing the function of the microRNA results in inhibition of the expression of the mRNA by at least 40%.

41. **(New)** The method of claim 33, wherein the step of testing the function of the microRNA results in inhibition of the expression of the mRNA by at least 60%.

42. **(New)** The method of claim 33, wherein the step of testing the function of the microRNA results in inhibition of the expression of the mRNA by at least 80%.

43. **(New)** The method of claim 29 wherein nucleic acid molecule synthesized in step d) comprises a sequence that when paired with the selected mRNA sequence was determined to have free energy of -30 kcal/mole or less.

44. **(New)** The method of claims 29 wherein the selected mRNA sequence is in the 3' untranslated region of an mRNA.
45. **(New)** The method of claim 29 wherein contacting the microRNA candidate oligonucleotide with the selected mRNA present in the cell results in inhibition of the expression of the mRNA by at least 20%.
46. **(New)** The method of claim 29 wherein contacting the microRNA candidate oligonucleotide with the selected mRNA present in the cell results in inhibition of the expression of the mRNA by at least 40%.
47. **(New)** The method of claim 29 wherein contacting the microRNA candidate oligonucleotide with the selected mRNA present in the cell results in inhibition of the expression of the mRNA by at least 60%.
48. **(New)** The method of claim 29 wherein contacting the microRNA candidate oligonucleotide with the selected mRNA present in the cell results in inhibition of the expression of the mRNA by at least 80%.